sequence (ProCre nucleic acid constructs) showed high levels of Cre-mediated recombination in the germ line, but did not show appreciable recombination in other tissues."; and at lines 23-28, please delete: "These data establish that ProCre nucleic acid constructs will facilitate the production of subtle, conditional, or tissue-specific mutations in mice as well as the production and analysis of mice with recombinase-conditional lethal alleles."

In the claims

Please cancel claims 11, 17, and 27 without prejudice.

Please amend claims 10, 13, 15, 25, 26, 28, 31, 32, 35, 36, 39 and 40-44 as follows. For the Examiner's convenience, claims pending and under consideration that are not amended herein are included and marked "Reiterated."

1. (Reiterated) A nucleic acid construct comprising a germ line-specific promoter operatively associated with a recombinase coding sequence.

2. (Reiterated) A nucleic acid construct according to claim 1 wherein said germline-specific promoter is the protamine 1 gene promoter, the protamine 2 gene promoter, the spermatid-specific promoter from the c-kit gene, the sperm-specific promoter from angiotensin-converting enzyme, oocyte specific promoter from the ZP1 gene, or oocyte specific promoter from the ZP2 gene or oocyte specific promoter from the ZP3 gene.

4. (Reiterated) A nucleic acid construct according to claim 1 wherein said recombinase coding sequence encodes Cre recombinase.

5. (Reiterated) A nucleic acid construct according to claim 4 wherein said construct is ProCre, comprising the protamine 1 gene promoter operatively associated with Cre recombinase.

6. (Reiterated) A nucleic acid construct according to claim 1 wherein said recombinase coding sequence encodes FLP recombinase.

8. (Reiterated) A nucleic acid construct according to claim 1 wherein said recombinase coding sequence encodes the R gene product of *Zygosaccharomyces*.

9. (Reiterated) A nucleic acid construct according to claim 8 wherein said construct is ProR, comprising the protamine 1 gene promoter operatively associated with the R gene product of *Zygosaccharomyces*.

10. (Amended) A nucleic acid construct comprising **[~~a conditional~~]** an inducible promoter operatively associated with a recombinase coding sequence.

12. (Reiterated) Embryonic stem cells containing a nucleic acid construct according to claim 1.

13. (Amended) Embryonic stem cells according to claim 12 wherein ~~the genome~~ thereof comprises ~~a transcriptionally active~~ selectable marker flanked by two recombinase recombination target sites.

14. (Reiterated) Embryonic stem cells according to claim 13 wherein the recombinase encoded by the recombinase coding sequence operatively associated with a germline-specific promoter is selective for the recombination target sites flanking said selectable marker.

4 15. (Amended) Embryonic stem cells according to claim 13 further comprising one or more of:

a nucleic acid fragment flanked by two recombinase recombination target sites, wherein said recombination target sites are different than the recombination target sites which flank said selectable marker,

a nucleic acid construct comprising **[~~a conditional~~]** an inducible promoter operatively associated with a recombinase coding sequence, or

a nucleic acid construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

16. (Reiterated) Embryonic stem cells containing a nucleic acid construct according to claim 2.

18. (Reiterated) Embryonic stem cells containing a nucleic acid construct according to claim 4.

19. (Reiterated) Embryonic stem cells containing a nucleic acid construct according to claim 5.

20. (Reiterated) Embryonic stem cells containing a nucleic acid construct according to claim 6.

21. (Reiterated) Embryonic stem cells containing a nucleic acid construct according to claim 7.

22. (Reiterated) Embryonic stem cells containing a nucleic acid construct according to claim 8.

23. (Reiterated) Embryonic stem cells containing a nucleic acid construct according to claim 9.

24. (Reiterated) Embryonic stem cells containing a nucleic acid construct according to claim 10.

25. (Amended) Embryonic stem cells according to claim 24 wherein the genome thereof comprises a transcriptionally active selectable marker flanked by two recombinase recombination target sites.

26. (Amended) Embryonic stem cells **[~~containing a nucleic acid construct according to claim 11~~]** comprising a tissue-specific promoter operatively associated with a recombinase coding sequence and further comprising a transcriptionally active selectable marker flanked by two recombinase recombination target sites in the genome of the stem cells.

28. (Amended) A method for **[~~excission~~]** excision of the transcriptionally active selectable marker from the embryonic stem cells of claim 13 said method comprising:

passaging the genome derived from said embryonic stem cells through gametogenesis to cause excision of the transcriptionally active selectable marker.

29. (Reiterated) A method according to claim 28 wherein said genome is passaged through spermatogenesis.

30. (Reiterated) A method according to claim 28 wherein said genome is passaged through oogenesis.

24 ~~31~~. (Amended) A method according to claim 21 ~~28~~ wherein said embryonic stem cells further comprise one or more of:

a nucleic acid fragment flanked by two recombinase recombination target sites, wherein said recombination target sites are different than the recombination target sites which flank said selectable marker,

a nucleic acid construct comprising **[~~a conditional~~]** an inducible promoter operatively associated with a recombinase coding sequence, or

a nucleic acid construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

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32. (Amended) A method for the production of recombinant alleles in a transgenic animal, said method comprising:

introducing a nucleic acid fragment flanked by ~~at least~~ two recombinase recombination target sites into embryonic ~~stem~~ cells according to claim 10, **[~~and~~]**

passaging the genome derived from said embryonic stem cells through gametogenesis ~~to obtain a transformed gamete; and~~

~~obtaining transgenic progeny from the transformed gamete.~~

33. (Reiterated) A method according to claim 32 wherein said nucleic acid fragment comprises an essential portion of a gene of interest.

34. (Reiterated) A method according to claim 32 wherein said nucleic acid fragment is introduced by homologous recombination, random insertion, retroviral insertion, or site specific-mediated recombination.

35. (Amended) A method for the production of recombinant alleles, said method comprising:

introducing a nucleic acid fragment flanked by at least two recombination target sites into embryonic stem cells according to claim 13, **[~~and~~]**

passaging the genome derived from said embryonic stem cells through gametogenesis, and

producing offspring resulting from crossing the genome of a gamete produced by the gametogenesis with the genome of a wild type animal,

whereby the nucleic acid fragment is inserted into the DNA of the offspring.

~~36~~. (Amended) A method according to claim ~~35~~ wherein said embryonic stem cells further comprise a second nucleic acid construct selected from the group consisting of a construct comprising **[~~a conditional~~]** an inducible promoter operatively associated with a recombinase coding sequence and a construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

37. (Reiterated) A method according to claim 36 wherein the recombinase encoded by said second construct is expressed in response to inducing conditions.

38. (Reiterated) A method according to claim 36 wherein the recombinase encoded by said second construct is expressed in a tissue selective manner.

~~39~~. (Amended) A method according to claim ~~35~~ wherein the recombination target sites flanking said nucleic acid fragment are recognized by a recombinase which is expressed under the control of **[~~a conditional~~]** an inducible promoter or a tissue specific promoter.

40. (Amended) A method for the production of recombinant alleles, said method comprising:

introducing at least one **[~~recombinase responsive~~]** construct according to claim 10 into embryonic stem cells **[~~according to claim 10~~]**,

wherein said **[~~construct(s) comprise(s)~~]** at least one construct further comprises a nucleic acid fragment flanked by a second pair of recombination target sites and a selectable marker**[~~,~~**

~~wherein said selectable marker is~~] flanked by a first pair of recombination target sites, **[~~and~~**

[~~wherein said nucleic acid fragment is flanked by a second pair of recombination target sites,~~]

passaging the genome derived from said embryonic stem cells selected for expression of the marker through gametogenesis to obtain a transformed gamete; and

obtaining first generation progeny containing the allele by crossing the genome of the transformed gamete with the genome of a wild type animal.

41. (Amended) A method according to claim 40 wherein said first pair of recombination target sites is recognized by a recombinase which is expressed under the control of a germline-specific promoter and said second pair of recombination target sites is recognized by a recombinase which is expressed under the control of **[~~a conditional~~]** an inducible promoter or a tissue specific promoter.

42. (Amended) A method according to claim 40 wherein said embryonic stem cells further comprise a second nucleic acid construct selected from the group consisting of a construct comprising **[~~a conditional~~]** an inducible promoter operatively associated with a recombinase coding sequence and a construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

43. (Amended) A method for the conditional assembly of functional gene(s) for expression in eukaryotic cells by recombination of individual inactive gene segments from one or more gene(s) of interest,

wherein each of said segments contains at least one recombinase recombination target site, and

wherein at least one of said segments contains at least two recombinase recombination target sites,

said method comprising:

introducing said individual inactive gene segments into an embryonic stem cell according to claim 10, thereby providing a DNA which encodes a functional gene of interest, the expression product of which is biologically active, upon passage of the genome derived from said stem cells through gametogenesis.

44. (Amended) A method for the generation of recombinant livestock, said method comprising:

combining a nucleic acid construct according to claim 1 with host pluripotential ES cells derived from early preimplantation embryos, **[~~and~~]**

introducing these embryos into a host female and

allowing the derived embryos to come to term.

REMARKS

The invention is based on the discovery that recombinase nucleic acid constructs can be expressed at high levels in the germ line but not to a functionally significant extent in either ES cells or embryonic or adult somatic tissues.